

in America with the settlers and as a result of the slave trade (7). To clarify the clinical outcomes of *M. lepromatosis* infection, a study in Mexico associated both mycobacteria with the forms already classified by Ridley and Jopling (8). That study found that, of the 55 cases with *M. lepromatosis* as the sole etiologic agent, 34 manifested LL, 13 developed diffuse LL, and the remaining 8 had other forms of leprosy. Fourteen patients carried both mycobacteria and showed all clinical forms (2). In contrast, 15% of leprosy patients in Brazil who had *M. lepromatosis* as the sole agent had polar tuberculoid leprosy, none had LL, and patients with infection by both mycobacteria had LL (7). The same study evaluated 8 patients in Myanmar and found *M. lepromatosis* in 2 patients, both of whom had LL (7).

This study demonstrates presence of *M. lepromatosis* in samples taken by our research group before 2008 when this mycobacterium was first reported (1). Therefore, we infer that *M. lepromatosis* has coexisted with *M. leprae* in Colombia for some time. Finally, this report confirms *M. lepromatosis* in Colombia. Genomic surveillance is needed to monitor the infection dynamics of both mycobacteria among leprosy patients and contacts to stop transmission and limit the dire physical, social, economic, and emotional consequences that these organisms cause among susceptible persons.

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Rare Case of Rickettsiosis Caused by *Rickettsia monacensis*, Portugal, 2021

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We report a case of rickettsiosis caused by *Rickettsia monacensis* in an immunocompetent 67-year-old man in Portugal who had eschar, erythematous rash, and an attached *Ixodes ricinus* tick. Seroconversion and eschar biopsy led to confirmed diagnosis by PCR. Physicians should be aware of this rare rickettsiosis, especially in geographic regions with the vector.

Rickettsia monacensis, spotted fever group rickettsiae (SFGR), are bacteria transmitted by *Ixodes* spp. ticks and are rarely reported as causing disease in humans. Few cases have been documented and laboratory confirmed (1–4). *R. monacensis* infection causing Mediterranean spotted fever (MSF)-like rickettsiosis was described in 2007 for 2 patients from La Rioja and the Basque Country, Spain, followed by 1 case in Italy (2012) and 2 cases in South Korea (2017 and 2019) (1–4). Despite the few human infections described, *R. monacensis* is frequently found (0.5%–42.5%) in *Ixodes ricinus* ticks in Europe, including Portugal and North Africa, and in another *Ixodes* species tick in Asia (3–5).

Three previously reported rickettsioses in Portugal were MSF caused by *R. conorii*, tick-borne lymphadenopathy caused by *R. slovaca*, and lymphangitis-associated rickettsiosis caused by *R. sibirica mongolitimonae* (6–8). We report *R. monacensis* infection in a human and *Rickettsia* in the attached tick.

In February 2021, a 67-year-old man with alcoholism-associated dilated cardiomyopathy and diabetes mellitus type 2 was hospitalized in Lisbon, Portugal. The patient reported a 5-day history of fever and appearance of rash on day 3 of fever onset. He lived in Lisbon and had traveled to a rural area 5 days before symptom onset. At admission, he had fever, fatigue, myalgia, and anorexia. Physical

examination showed disperse upper-body erythematous exanthema, palmo-plantar erythema, and an eschar surrounded by erythema on his upper left back (Figure). An engorged female *I. ricinus* tick was removed from the patient. Laboratory evaluation showed hematologic, hepatic, and renal abnormalities; anemia (hemoglobin 9.7 g/dL); lymphopenia (420 cells/ μ L); thrombocytopenia (38,000 platelets/ mm^3); and increased serum levels of creatinine (2.23 mg/dL), alanine aminotransferase (73 IU/L), aspartate aminotransferase (89 IU/L), creatine phosphokinase (116 IU/dL), lactate dehydrogenase (148 IU/L), and C-reactive protein (159.5 mg/L). Electrocardiography findings were unremarkable. Oral doxycycline (200 mg/d) was empirically started on hospitalization day 1.

After the patient had been hospitalized for 12 hours and received 1 dose of doxycycline, we biopsied the eschar and collected a blood sample. PCR and DNA sequence analysis of partial fragments of *ompA* and *gltA* genes from the tick and biopsy samples showed 100% identity with nucleotide sequences of *R. monacensis* (GenBank accession no. LN794217). Screening for *Borrelia* DNA in the tick was negative.

For antibody testing we used an immunofluorescence assay from FOCUS Diagnostics (<https://www.focusdx.com>), which used commercial *R. conorii* IFA substrate slides for IgG and IgM; results demonstrated seroconversion within 2 weeks in consecutively collected samples. We detected no antibodies in the acute-phase serum sample collected on day 6 after symptom onset, and we detected reactive antibodies against SFGR (IgM titer 32, IgG titer 128) in the second sample only, collected 3 weeks after illness onset (9). Supplemental methods and results are in the Appendix (<https://wwwnc.cdc.gov/EID/article/28/5/21-1836-App1.pdf>).



Figure. Patient with rickettsiosis caused by *Rickettsia monacensis*, Portugal, 2021. A) Rash and eschar; B) rash on soles; C) rash on palms.

Table. Clinical features and laboratory diagnosis of patients with *Rickettsia monacensis* infection, 2003–2021

Feature	Patient 1, La Rioja, Spain (1)	Patient 2, Basque, Spain (1)	Patient 3, Sardinia, Italy (2)	Patient 4, South Korea (3)	Patient 5, South Korea (4)	Patient 6, Portugal (this study)
Epidemiologic						
Age, y/ sex	84/M	59/F	28/M	73/M	75/F	67/M
Date of onset	Jun 2003	Sep 2003	Apr 2011	2006	Oct 2019	Feb 2021
Tick bite history	NK	Yes	NK	NK	NK	Yes
Clinical						
Fever, °C (°F)	Yes, 39.5° (103.1°)	Yes, 38° (100.4°)	Yes, 38.2° (100.8°)	Yes, 40° (104.0°)	Yes, 38.4° (101.1°)	Yes, 39.9° (103.8°)
Eschar (location)	No	No	Yes (calf)	Yes (back)	Yes (scalp)	Yes (back)
Rash	Yes	Yes	No	Yes	Yes	Yes
Type	Maculopapular	Erythematous		Maculopapular	Maculopapular	Erythematous
Including palm and soles	Yes					Yes
Headache	Yes	Yes	Yes	Yes	NK	Yes
Lymphadenopathy	NK	NK	NK	Yes	Yes	NK
Laboratory test results						
SFGR IFA titer						
Sample 1	<40 IgG	2,560 IgG	128 IgG	320 (IgM + IgG)	<16 IgM/32 IgG, negative	Negative
Sample 2	1,280 IgG (26 wk later)	1,280 IgG	NA	NA	16 IgM/128 IgG (2 mo)	32 IgM /128 IgG (2 wk)
Culture, blood/biopsy	Positive	Negative	NA	Positive	NA	NA
PCR detection, blood and/or skin biopsy	Positive	Positive	Positive	NK	Positive	Positive
Co-infections with other pathogens	NK	NK	NK	NK	<i>Orientia tsutsugamushi</i>	NK
Treatment						
Hospitalization	NA	NA	Yes	Yes	Yes	Yes
Antimicrobial drug	Doxycycline 100 mg every 12 h for 10 d	Doxycycline 100 mg every 12 h for 10 d	Doxycycline 100 mg every 12 h for 7 d	Azithromycin 500 mg, 1 dose	Doxycycline 200 mg/d	Doxycycline 200 mg/d

After 48 hours of antimicrobial therapy, the patient was afebrile; after 4 days, exanthema was completely resolved; and after 7 days, all symptoms had resolved. The patient was discharged and scheduled for outpatient follow-up.

We confirm that *R. monacensis* caused disease in this patient. Very few cases of human infection with *R. monacensis* have been reported, possibly because this species is not considered to be very pathogenic and for most patients might cause self-limited infection (1–5). Another hypothesis is that cases have been misdiagnosed or confirmed by serology only, which cannot distinguish among SFGR species (8,9). Moreover, if cases occur in the autumn/winter, when adult *I. ricinus* ticks are more active and outside the peak season (June–September) for MSF, some physicians might not think of rickettsiosis as the cause, particularly if there is no epidemiologic context and clinical findings are not highly suggestive.

For the patient reported here, we identified an eschar, as was done for the 3 other patients from Italy and South Korea (Table). However, the first 2 patients identified in Spain did not have any sign of an eschar. We are unaware whether any specific patient

host factors could be associated with *R. monacensis* infection, but alcoholism in the patient reported here could have been a risk factor for severity (8). With exception of the patient from Italy, all patients were >59 years of age, including the patient from Portugal, and at least 3 were hospitalized. In general, it would seem that older persons are more susceptible to disease, even when infected with low-pathogenicity *Rickettsia*. For instance, in the case report of an 8-year-old child from Croatia with Lyme borreliosis, in whom *R. monacensis* DNA was also detected in a skin biopsy of the erythema migrans tissue, antibodies against *Borrelia* were detected but not antibodies against SFGR (10).

This case of infection with *R. monacensis*, formerly considered to be of low pathogenicity and found in *Ixodes* spp. ticks, was associated with disease in an immunocompetent patient. Other cases may be underdiagnosed, particularly outside the usual summer months when MSF cases peak in Portugal. Moreover, because *R. monacensis* shares the same vector as *Borrelia* spp. and these co-infections have been detected, physicians should be aware of this rickettsiosis, especially in areas where the vector is present.

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Domestic Dogs as Sentinels for West Nile Virus but not *Aedes*-borne Flaviviruses, Mexico

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We tested 294 domestic pet dogs in Mexico for neutralizing antibodies for mosquito-borne flaviviruses. We found high (42.6%) exposure to West Nile virus in Reynosa (northern Mexico) and low (1.2%) exposure in Tuxtla Gutiérrez (southern Mexico) but very limited exposure to *Aedes*-borne flaviviruses. Domestic dogs may be useful sentinels for West Nile virus.

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Appendix

Methods

DNA was extracted from skin biopsy and tick using a MagCore HF 16 Plus automated nucleic acid extractor (RBC Bioscience, Taiwan) and MagCore® Genomic DNA tissue kit.

For tick molecular identification a conventional PCR targeting partial region of mitochondrial marker 16S ribosomal RNA gene using the primer set 16S+1/16S-1, which amplifies approximately 456 bp, was performed as previously described by Black & Piesman (1).

PCR targeting the rickettsial genes for citrate synthase (*gltA*) and outer membrane protein A (*ompA*) were performed with specific primers. For the amplification of skin biopsy DNA we have used a nested-PCR using the primer sets RpCS.415/ RpCS.1220 and RpCS.877/RpCS.1258n to amplify partial fragment of citrate synthase (*gltA*) gene and primer sets Rr190.70p/Rr190.701n and Rr190.70p/190.602n to amplify a fragment of *ompA* gene, according previously described by Portillo and collaborators (2). For tick DNA amplification we have used regular conventional PCR using the RpCS.415/ RpCS.1220 and Rr190.70p/190.602n primer sets of *gltA* and *ompA* genes (2).

PCR were performed in a 50-μL reaction mixture containing 25 μL of the High Fidelity PCR Master Kit buffer (Roche Diagnostics, GmbH, Mannheim, Germany), 2 μL of each primer at 0.2 μmol/L, and 10 μL genomic DNA. Amplification was performed in a DNA thermocycler (T-3 thermoblock T, Biometra, Goettingen, Germany) according previous described amplification protocols (2). For each reaction, a negative control (water) and a positive control of *R. rickettsii* were included. Five microliters of the PCR products were resolved by electrophoresis in 1.2% agarose gel with GelRed® Nucleic Acid Gel Stain (Biotium, California,

USA) and examined by UV transillumination. PCR products of the expected size were purified with ExoSAP-IT™ PCR Product Cleanup Reagent and sequenced in an ABI automated sequencer (Applied Biosystems, Foster City, CA, USA) by using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems), according to the protocols supplied by manufacturers. All sequences were determined by the consensus of the forward and reverse sequence analysis. The sequences of the *gltA*, *ompA*, amplicons were aligned with the corresponding sequences of other *Rickettsia* species available in GenBank/EMBL database, by using BLASTN software.

We also have extract the DNA from the tick and did PCR for the tick mitochondrial marker 16S ribosomal RNA gene using the primer set of Black and Piesman (1) to confirm the *Ixodes ricinus* species. We have add the GenBank accessing number of our sequence (OK484994).

Results

The sequence from the skin biopsy designated PoHuR34655 exhibited nucleotide sequence of *gltA* (341/341 bp) and *ompA* (488/488bp) 100% identical to *Rickettsia monacensis* IrMunich strain (LN 794217). The tick sequence designated PoTiR20 was also 100% (766/766 bp) identical to *gltA* of *Rickettsia monacensis* IrMunich strain (LN 794217). For *ompA* the sequence of the tick is identical and have the same length (bp) to the sequence of the biopsy specimen. The sequence which identified the tick as *Ixodes ricinus* was designated PTtick 20.

Nucleotide Sequence Accession Numbers

The GenBank nucleotide sequence accession numbers for partial sequences of *gltA*, *ompA*, genes generated in this study for PoHuR34655 are: OK504620, OK504619, respectively; *gltA* partial sequence for PoTiR20 is OK504621. GenBank accessing number for PTtick20 : OK484994 (*I.ricinus* 16S rDNA).

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